

AMENDMENTS TO THE SPECIFICATION

Kindly amend the paragraph starting at page 5, line 10 of the English language specification as follows.

This oligonucleotide is preferably formed by a sequence coding for more than 2 (P-K) units. Preferably, n is 30 or less (SEQ ID NO:22), in particular below 20 and advantageously, n equals 4 (SEQ ID NO:13), 5 (SEQ ID NO:14), 6 (SEQ ID NO:15), 7 (SEQ ID NO:16), 8 (SEQ ID NO:17), 9 (SEQ ID NO:18), or 10 (SEQ ID NO:19), or 15 (SEQ ID NO:20).

Kindly amend the paragraph starting at page 6, line 13 of the English language specification as follows.

As an example, a preferred oligonucleotide of the invention is characterized in that it codes for a polypeptide with formula (P-K), formula K-(P-K)₄ (SEQ ID NO:21), or with formula 2K(P-K)₄ (SEQ ID NO:23).

Kindly amend the paragraph starting at page 20, line 9 of the English language specification as follows.

Two synthetic nucleotides with the following sequences: SEQ ID N° 1: 5'CGATGAATTCAAACCAAAGCCAAAGCCGAAGCCAAAAGAATTCA3', and the inverse sequence termed SEQ ID N° 2, with the following sequence: 5'AGCTTGAATTCTTTGGCTTCGGCTTGGCTTGTTGAATTCA3' coding for lysine-rich sequences termed (P-K)₄ (SEQ ID NO:13), were hybridised, digested with EcoRI and cloned in a an EcoRI site of pHbP2 and pPbP4. Three clones were selected:

pPo2 and pHo3 containing the sequence coding for K(P-K)₄ (SEQ ID NO:21) and pHo4 comprising the truncated form of the sequence coding for γ -zein containing a tandem 2K(P-K)₄ (SEQ ID NO:23) (in the form of a sequence K(P-K)₄ EF K(P-K)₄ (SEQ ID NO:24)) of the lysine-rich coding sequence. The same hybridised oligonucleotides were digested with Clal-HindIII enzymes and cloned in plasmid pNaN1 restricted using the same enzymes. The selected clone, pNol, contained the sequence coding for the lysine-rich sequence K(P-K)₄ (SEQ ID NO:21) at the N-terminal extremity of the corresponding modified γ -zein.

Kindly amend the paragraph starting at page 24, line 11 of the English language specification as follows.

The inventors have now constructed modified γ -zein genes by introducing synthetic oligonucleotides coding for lysine-rich sequences into different sites of the γ -zein coding sequence. Modified γ -zein constructs were created so as to avoid placing lysine-rich coding sequences in domains constituted by the tandem repeat and the cysteine-rich domain. Modifications of the γ -zein coding sequence were made in the sequence corresponding to the Pro-X domain. Further, to minimise any alteration to protein folding, the lysine-rich sequences (P-K)_n were defined to imitate the sequence of the Pro-X domain. As can be seen in Figure 3, a sequence K(P-K)₄ (SEQ ID NO:21) has been introduced into the protein P20 γ Z after the Pro-X region and in protein H30 γ Z and in protein H45 γ Z, amino acid sequences including K(P-K)₄ (SEQ ID NO:21) and 2K(P-K)₄ (SEQ ID NO:23) (in the form of a sequence K(P-K)₄ EF K(P-K)₄ (SEQ ID NO:24)) respectively replace the Pro-X domain of the γ -zein (γ Z, fig, 3). To study whether the C-terminal extremity was a neutral site for the introduction of lysine-rich sequences, a supplemental N13 γ Z protein was created by inserting a sequence containing K(P-K)₄ (SEQ ID NO:21) five amino acids upstream of the C-terminal extremity (Figure 3).

Kindly amend the paragraph starting at page 28, line 1 of the English language specification as follows.

Figure 6A (line 2) shows that no trace of the N13 γ Z protein could be detected, indicating that the corresponding chimera gene was not expressed in the endosperm cells or that the N13 γ Z protein had degraded. The RNAs of endosperms transformed with the DNAs coding for the H45 γ Z and N13 γ Z proteins and the RNAs of non transformed endosperms were analysed. From the total RNAs, the cDNAs were prepared and amplified by PCR using specific primers. Figure 6B shows the Southern blot analysis of three cDNA samples hybridised with an oligonucleotide coding for a sequence K(Pro-Lys)₄ (SEQ ID NO:21) used as a probe. The results indicated that the N13 γ Z gene was correctly expressed (Figure 6B, line 3). The presence of bands in the H45 γ Z and N13 γ Z samples but not in the non transformed endosperms, has suggested that the N13 γ Z protein was degraded during the 24 hours of incubation. From these observations, the inventors have concluded that the insertion site for lysine-rich sequences was critical for the stability of the modified γ -zein.

Kindly amend the paragraph starting at page 34, line 27 of the English language specification as follows.

Whole transgenic plants, selected in a medium containing kanamycin, were homogenised in liquid nitrogen. The transgenic proteins were selectively extracted with a solution containing ethanol/0.125 N hydrochloric acid HCl in a proportion of 3:1 (v/v) with 5% of mercaptoethanol and protease inhibitors. The proteins extracted with this solution were precipitated in 5 volumes of acetone and analysed by SDS-PAGE and immunoblotting. The protein extracts from non transgenic plants were used as controls. The proteins resulting from insertion of K(P-K)₄ (SEQ ID NO:21) sequences in the γ -zein were properly expressed in Arabidopsis thaliana plants using the constitutive promoter 35S from CaMV. On the immunoblots, antibodies α G2 and α PL recognised

electrophoresis bands corresponding to proteins P20 γ Z and H30 γ Z. These bands migrated with apparent molecular weights in accordance with those which were previously observed in the in-vitro translation/translocation experiments (30 kD and 26 kD respectively). As observed in the transgenic Arabidopsis plants expressing γ -zein (Geli et al., Plant Cell 6: 1911-1922 (1994)), the proteins P20 γ Z and H30 γ Z migrated in the form of two electrophoresis bands, namely the bands corresponding to 36 and 30 kD for P20 γ Z and the bands corresponding to 32 and 26 kD for H30 γ Z. The higher bands could correspond to products which have undergone post-translational modifications. Such a post-translational modification was not detected in the transformed maize endosperms. This result suggests that the modification would appear when these proteins are expressed in a heterologous system such as Arabidopsis thaliana.

Please replace the current sequence listing with the one submitted with the concurrently filed Statement under 37 C.F.R. § 1.825.